Detailed TALEN construction methods

Two successive rounds of Golden Gate cloning assembly were used to generate RVD repeat module TALEN expressing plasmids. In the first round of Golden Gate cloning two separate arrays corresponding to repeat modules 1–10 and 11-(n-1) were assembled into intermediate vectors and screened on IPTG/X-gal plates for those acquiring RVD arrays. Confirmation of correct assembly was confirmed by XbaI and AflII restriction enzyme digestion followed by sequencing. In the second round of Golden Gate cloning these two arrays (RVDs 1-10 and RVDs 11-(n-1)) and sequences encoding the nth motif were assembled into the final expression backbone vector to generate either left or right TALEN expressing plasmids, followed by screening on IPTG/X-gal plates. Confirmation of correct assembly was determined first by SphI and BamHI restriction enzyme digestion followed by sequencing.

Two versions of the final backbone vector were used in this report: pCS2TAL3 (1) and the pCS2TAL3-PUb. pCS2TAL3 contains the simian IE94 cytomegalovirus eukaryotic enhancer/promoter (CMV), the prokaryotic SP6 RNA polymerase recognition sequence, and the SV40 polyadenylation signal sequence (SV40pA) derived from the pCS2 vector. A nuclear localization signal (NLS); either a FLAG or HA epitope; and truncated TAL protein N-terminus and C-terminus (TAL-N9 and TAL-C9) sequences were derived from pTAL3 (2). The CMV promoter in the pCS2TAL3 TALEN expression vector was replaced by the *Aedes PUb* promoter from the pSLfa-*PUb*-MCS vector by Gibson Cloning using the primers listed below. The new vector, pCS2TAL3-*PUb*, is identical to pCS2TAL3 expect for the promoter used and was used in the second cloning step above. Several versions of the FokI endonuclease domain were compared to each other: WT (HA and Flag tagged), DD and RR, and DDD and RRR. The *Aedes PUb* promoter was Gibson cloned into the pCS2TAL3 backbone of each FokI version.

Primers used in Gibson cloning

PUb-ProClon-F 5’- ttggcaagtacattactaTATCTTTACATGTAGCTTGTGCATTGAATCC-3’

PUb-ProClon-R 5’-ccaggcagaatggcgGTTGAAATCTCTGTTGAGCAGAAAAAGAAAC-3’

pCS2TAL3-ProClon-F 5’-CGCCATTCTGCCTGGGGAC-3’

pCS2TAL3-ProClon-R 5’TAGTAATGTACTTGCCAAGTTACTATTAATAGATATTG-3’

Addgene numbers pCS2TAL3 vectors: DDD,48637; RRR, 48636; DD, 37275; RR, 37276; Golden Gate TALEN Kit #1000000024

Complete list of vectors made by the MGD Core:

ago2

pCS2TAL3-ago2-exon2-DDD

pCS2TAL3-ago2-exon2-RRR

pCS2TAL3-*PUb*-ago2-exon2-WT-Flag

pCS2TAL3-*PUb*-ago2-exon2-WT-HA

pCS2TAL3-ago2-exon3-DDD

pCS2TAL3-ago2-exon3-RRR

pCS2TAL3-*PUb*-ago2-exon3-WT-Flag

pCS2TAL3-*PUb*-ago2-exon3-WT-HA

dcr2

pCS2TAL3-dcr2-exon5-DDD

pCS2TAL3- dcr2-exon5-RRR

pCS2TAL3-*PUb*- dcr2-exon5-DDD

pCS2TAL3-*PUb*- dcr2-exon5-RRR

pCS2TAL3-*PUb*- dcr2-exon5-DD

pCS2TAL3-*PUb*- dcr2-exon5-RR

pCS2TAL3-*PUb*- dcr2-exon5-WT-Flag

pCS2TAL3-*PUb*- dcr2-exon5-WT-HA

pCS2TAL3-dcr2-exon8-DDD

pCS2TAL3- dcr2-exon8-RRR

pCS2TAL3-*PUb*- dcr2-exon8-WT-Flag

pCS2TAL3-*PUb*- dcr2-exon8-WT-HA

lig4

pCS2TAL3-lig4-A-DDD

pCS2TAL3-lig4-A-RRR

pCS2TAL3-lig4-B-DDD

pCS2TAL3-lig4-B-RRR

kmo

pCS2TAL3-*PUb*-kmoA-DDD

pCS2TAL3-*PUb*-kmoA-RRR

pCS2TAL3-*PUb*-kmoA-DD

pCS2TAL3-*PUb*-kmoA-RR

pCS2TAL3-*PUb*-kmoA-WT-Flag

pCS2TAL3-*PUb*-kmoA-WT-HA

pCS2TAL3-*PUb*-kmoB-DDD

pCS2TAL3-*PUb*-kmoB-DD

pCS2TAL3-*PUb*-kmoB-WT-Flag

Detailed Plasmid Construction Methods

To generate the donor vectors for *kmo*, pSLfa-*PUb*GFP-*kmo*-HR-2kb, a ~2200 bp genomic fragment upstream of the *kmo* TALEN target site was amplified with Platimun Pfx polymerase (Invitrogen, Carlsbad, CA) using primers 5'-ttttctcgagCAATAGGCAAAACTGATTTTAATAATAACG-3' and 5'-ttttccgcggCATGGCAGTGACGTCGATAGGAT-3' (94°C 2 min, 35 cycles of [94°C 10 sec, 56°C 30 sec, 68°C 4 min 30 sec] 68°C 10 min). A 1723 bp downstream amplicon was amplified using the primers 5'-GAGAAATACTATTTGTTAAGCACTG-3' and 5'-ttttgaattcCCGTAGAAGGGAACCAT-3' (94°C 2 min, 35 cycles of [94°C 10 sec, 52°C 30 sec, 68°C 2 min 40 sec] 68°C 10 min). Amplicons were gel-extracted and digested with EcoRI or SacII/XhoI prior to cloning into the corresponding sites of pSLfa-*PUb*EGFP-MCS.

To generate the donor vector for *dcr2*, pSLfa-*PUb*GFP-*dcr2*-HR, a 1063 bp genomic fragment 5' of the *dcr2* TALEN target site was amplified using primers 5'-ttttccgcggTGTCCTGTTTCCAAGCATCCAC-3' and 5'-ttttctcgagAGCTGACTCTGTTGCCTCCTCTACG-3'. Likewise, a 1717 bp genomic fragment 3'-of the *dcr2* TALEN target site using primers 5'-ttttgaattcTATGCACGGGATTATGGAACAATTTCTGAG-3' and 5'-ttttggatccACACATCTTTCAAATTGTTAAGCTGGTA-3'. PCR conditions for both reactions were (94°C 2 min, 35 cycles of [94°C 10 sec, 58°C 30 sec, 68°C 3 min] 68°C 10 min). Amplicons were gel-extracted using NucleoSpin Gel and PCR clean-up kit (Machery-Nagel, Bethlehem, PA), digested with SacII/ XhoI (5' fragment) and EcoRI/BamHI (3'fragment) prior to cloning into the corresponding sites of pSLfa *PUb*EGFP-MCS (3).

To generate the donor vector pSLfa-*PUb*GFP-*kmo*-HR-500bp, a ~2500 bp genomic fragment upstream of the KMO TALEN target site was first amplified using primers 5'-AGTGAAATTACTGAAGTCA-3' and 5'- ttttacgcgtCATGGCAGTGACGTCGATAGGAT-3'; this amplicon was used as a template for a second round of PCR with primers 5'-ttatttgaggaggtttctctgtaaataatgCGTCGTCCGAATTAGTAGTT-3' and 5'-ttttacgcgtCATGGCAGTGACGTCGATAGGAT-3' in order to add an I-AniI recognition site. Re-amplification with the primers 5'- ttttctcgagTTATTTGAGGAGGTTTC-3' and 5'-ttttacgcgtCATGGCAGTGACGTCGATAGGAT-3' added an XhoI site, PCR conditions: (94°C 2 min, 35 cycles of [94°C 10 sec, 50°C 30 sec, 68°C 3.5 min] 68°C for 10 min) using Pfx. A 503 bp amplicon was obtained using the primers 5'-ttttgaattcCCGTAGAAGGGAACCAT-3' and 5'-ttatttgaggaggtttctctgtaaataatgTCCAACGACGAAGGAATCTACTC-3', which was re-amplified with the primers 5'-ttttgaattcCCGTAGAAGGGAACCAT-3' and 5'-ttttgaattcTTATTTGAGGAGGTTTCTCTGT-3', PCR conditions: (94°C 2 min, 35 cycles of [94°C 10 sec, 52°C sec, 68°C 2 min 40 sec] 68°C 10 min). Final amplicons were then gel-extracted and digested with EcoRI and MluI/XhoI prior to cloning into the corresponding sites of pSLfa-*PUb*GFP-MCS.

The non-homologous end joining construct was generated by digesting the pGL3-*PUb*-FF (3), with BsrGI. Oligonucleotides corresponding to a Y2-I-AniI homing endonuclease target site (ZA2355: 5'GTACATTATTTACAGAGAAACCTCCTCAAAT3' and ZA2356: 5'GTACATTTGAGGAGGTTTCTCTGTAAATAAT3') were annealed and ligated into the BsrGI site of the linearized plasmid, using T4-DNA ligase (Promega, Madison, WI). The insertion generates a frameshift within the firefly ORF.

dsRNA preparation.

Ae. aegypti cDNA was used as template for PCR for *ku70*, *ku80* and *lig4* using the oligonucleotide primers listed [see "List of dsRNA primers" later in Appendix]. EGFP DNA template was generated by PCR from pMos-*3xP3*.DsRed-*Ub*$_{L40}$.EGFP-*attP* plasmid, previously described (3). DNA templates (*ku70*#1, *ku80* and EGFP) were amplified using Phusion polymerase (NEB, Madison, WI) with the following conditions:98°C 1 min, 35 cycles of [98°C 10 sec, (53°C 30 sec for *ku70*#1 and *ku80*, 48°C 30 sec for EGFP), 72°C 40 sec], 72°C 10 min. DNA templates (*ku70*#2 and *lig4*) were amplified using Pfx polymerase: 94°C 30 sec, 35 cycles of [94°C 10 sec, 56°C 30 sec, 68°C 40 sec], 68°C 10 min. All amplicons were purified using the NucleoSpin Gel and PCR clean-up kit. From each amplicon, dsRNAs were prepared using the Replicator RNAi Kit (Thermo Scientific, Lafayette, CO). A sample (0.5 µl) of each reaction was analyzed by gel electrophoresis for integrity and size**.** After nuclease treatment (2 µl DNaseI and 2 µl RNaseA), the dsRNA was purified using MEGAclear Transcription Clean-Up kit (Ambion, Invitrogen, Life Technologies, Grand Island, NY). Following purification**,** 0.5 µl of the dsRNA was again visualized using gel electrophoresis, aliquoted and stored at -80°C.

Detection of off-target effects by HRMA.

The most highly active sgRNA of each gene investigated, as determined by embryonic assay, was analyzed for off-target analysis by HRMA. Each target sequence (23nts) was analyzed using the flyCRISPR target finder tool (with maximum stringency and 'NGG' only parameters) (4). Using the ranked output, the two most likely off target areas were identified and HRMA primer assays were designed (parameters previously described for using the Lightscanner Primer Design

software). A complete list of HRMA primers used is presented later in the appendix "List of HRMA assay primers". Off target HRMA was conducted using the same samples of LVP embryos that had been injected previously with that specific sgRNA. PCR conditions and analysis replicated the previously described protocol for HRMA assays.

Supplemental References:

1. Dahlem TJ, *et al.* (2012) Simple Methods for Generating and Detecting Locus-Specific Mutations Induced with TALENs in the Zebrafish Genome. *PLoS Genet* 8(8):e1002861.
2. Cermak T, *et al.* (2011) Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Res* 39(12):e82.
3. Anderson MA, Gross TL, Myles KM, & Adelman ZN (2010) Validation of novel promoter sequences derived from two endogenous ubiquitin genes in transgenic *Aedes aegypti*. *Insect Mol Biol* 19(4):441-449.
4. Gratz SJ, *et al.* (2014) Highly specific and efficient CRISPR/Cas9-catalyzed homology-directed repair in *Drosophila*. *Genetics* 196(4):961-971.
5. Crooks GE, Hon G, Chandonia JM, & Brenner SE (2004) WebLogo: a sequence logo generator. *Genome research* 14(6):1188-1190.

List of crRNA and sgRNA target sequences.

Name	Target sequence	PAM site	Detectable mutation by HRMA[a]	LS max height[b]
kmo-281*	ATACTGTTGAAGTTGGTGAA	CGG	y	0.4
kmo-288*	ACTTAATACTGTTGAAGT	TGG	y	0.047
kmo-304	TTCAACAGTATTAAGTGCGA	TGG	y	0.015
kmo-447	GCCATATAATGTGGGCGGCA	AGG	y	0.1
kmo-460	CGGCAAGGCGGTGATCAT	TGG	y	0.42
kmo-468	CGGTGATCATTGGTGATG	CGG	y	0.32
kmo-519*	CACAGTACAATCCTCGAATC	CGG	y	0.22
kmo-519	GGGAATGAATGCCGGATTCG	AGG	n	0.04
ku70-1*	CAGCTCTATTTCATCCGCAG	GGG	y	0.048
ku70-2*	TCCAGCTCTATTTCATCCGC	AGG	n	0.012
ku70-3	AACGATGAGCCGCATTCGGC	CGG	y	0.27
ku70-4	GCAGTGTTTTGTCAAGGCGA	AGG	y	0.04
lig4-1	ACCGGTAGCAATGGGAGCAG	CGG	n	0.018
lig4-2	GAATCCGGCACCGGTAGCAA	TGG	n	0.025
lig4-3*	CCAGCTTGAGATCTACTCTT	TGG	y	0.04

lig4-4*	TGGCAAAGCATGGGCTTTAC	AGG	n	0.005
lig4-5	GAGGCGGTCGAGAGTGGCGA	AGG	n	0.028
lig4-6	GGGGCTGTATCATCCCAAAG	CGG	n	0.02
lig4-7	GATGCGCGGACGTTGCCCGG	AGG	y	0.11
lig4-8	CAACCAGCACCTGGATGCCA	TGG	n	0.017
lig4-9	CCAAATTCATAAAGAAGGAC	AGG	n	0.07
lig4-10	AGGTTCCAAATTCATAAAGA	AGG	n	0.00
loqs-1	GGTCGAGGATCTGAACGGAC	TGG	y	0.027
loqs-2	GGACTGGTATCCGGTGACAA	CGG	y	0.03
loqs-3*	AGATTCGGTTGACCGACCGG	GGG	y	0.2
loqs-4	GCCCTCAAGACCGAGCTGAC	GGG	y	0.05
loqs-b1*	GCCGTGACCGGTAGTGAGGC	TGG	n	0.018
loqs-b2	AAGGCCCTGAAGGCACGCAG	CGG	y	0.03
loqs-b3	AGGCGGCCAGCATTTCCGGG	CGG	y	0.02
loqs-b4*	CGTATTCCCAGCCTCACTAC	CGG	y	0.007
loqs-b5	AGCATTTCCGGGCGGTACGC	TGG	y	0.03
loqs-b6*	CTTCAATCCAGCGTACCGCC	CGG	y	0.079

loqs-b7	AAAAGTGTCCCAGTTCCACA	AGG	y	0.014
loqs-b8	CCAGTTCCACAAGGCCCTGA	AGG	y	0.022
r2d2-1	CGGAATTGGCCGGTCAAAGA	AGG	n	0.022
r2d2-2	ACCACTTCGGTTTTGGCCCT	CGG	n	0.065
r2d2-3*	TCCGCTGCTGGTGAATCCGA	GGG	y	0.03
r2d2-4*	GGAGTCTTCGTGCTCAGGAC	TGG	y	0.009
r2d2-5*	CGTGCTCAGGACTGGCTTTG	AGG	y	0.005
r2d2-6	GAGCACGAAGACTCCAATAA	CGG	y	0.082
r2d2-7	TCACCAGCAGCGGAATTGGC	CGG	n	0.011
r2d2-8*	TCAAACTCTGGAGCATGCGA	AGG	y	0.005
r2d2-9*	TCGTCTTGTGAATTCCCCGG	CGG	y	0.017
r2d2-10*	GTTTGGAATCCTTCTTTGAC	CGG	y	0.017
nix-1*	GCAGGTTTAACGTCGCAATG	TGG	n	0.074
nix-2*	GAACGATATCGAACAATTGC	AGG	n	0.007
nix-3*	GTCAGCAGATATCTTGAATA	AGG	y	0.005
nix-4*	TACCACAATGCAAGTATCAT	AGG	n	0.006
nix-5	GAACGAAACATTAACATGTG	CGG	y	0.23

nix-6*	CGGTCTAAATTCAGAAAGCC	AGG	y	0.03
nix-7*	TTAGATTTGTGGAGAAATCC	TGG	n	0.00
nix-8	CTTGAATAAGGCAAACTTAG	TGG	y	0.26

Underlined letters denote predicted cleavage site; *denotes cleavage site of non-transcribed strand.

[a] (Yes, y; No, n)Mutation detection was strongly dependent on the biological variation amongst control and experimental samples, in addition to the LS peak height. Thus, where experimental variation was extremely small, the sensitivity of the HRMA assay to detect low-activity sgRNAs was increased.

[b] Maximum value of the difference curve (Δ Fluorescence) between the experimental sample and controls using the LightScanner Call-IT 2.0 analysis software.

List of TALEN target sites.

TALEN	TALE-Binding Site (Left)	Spacer	TALE-Binding Site (Right)
lig4-exon2-A	T CCTGTAAAGCCCATGCT	ttgccaaagagtag	ATCTCAAGCTGGTGGATGGCA
lig4-exon2-B	T CGACAAAAGTGAGCTAATCT	atcgagataaaagcg	AGAACACTGATGTCAAGGCA
dcr2-exon5	T CTCGGTTAAACACATCAACT	tgcttatatttgatga	ATGCCACCATGGTGTAGGAGA
dcr2-exon8	T AGACTGCAAATACCAGCT	taacaatttgaaag	ATGTGTATTTCCGTCATTGGA
ago2-exon2	T CGCATTCGTCGTCACCGT	cgcatgctgcggccctcg	AGCGGGTTGAGGAGGA
ago2-exon3	T CCTACCGCAATGACCGT	agggtaaaaccaatg	AGGGGAGTTTGGCGGGCTGACA
kmo MM	T GAATGCCGGATTCGAG#	gattgtactgtgttg	ACCGAGTTGTTCAATCA
kmo PM	T GAATGCCGGATTCGAG##	gattgtactgtgttg	ACCGAGTTGTTCAATCA

Final RVD was specified as NG (mismatched, MM). ## Final RVD was specified as NN (perfect match, PM)

List of oligonucleotides used to generate sgRNAs.

sgRNA	Primer	Sequence[a]
sgRNA rev	ZA2267	AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC
KMO288*	ZA2268	GAAATTAATACGACTCACTATAGGACTTAATACTGTTGAAGTGTTTTAGAGCTAGAAATAGC
KMO447	ZA2368	GAAATTAATACGACTCACTATAGGGCCATATAATGTGGGCGGCAGTTTTAGAGCTAGAAATAGC
KMO468	ZA2269	GAAATTAATACGACTCACTATAGGCGGTGATCATTGGTGATGGTTTTAGAGCTAGAAATAGC
KMO460	ZA2270	GAAATTAATACGACTCACTATAGGCGGCAAGGCGGTGATCATGTTTTAGAGCTAGAAATAGC
KMO304	ZA2369	GAAATTAATACGACTCACTATAGGTTCAACAGTATTAAGTGCGAGTTTTAGAGCTAGAAATAGC
KMO281*	ZA2370	GAAATTAATACGACTCACTATAGGATACTGTTGAAGTTGGTGAAGTTTTAGAGCTAGAAATAGC
KMO519*	ZA2354	GAAATTAATACGACTCACTATAGGCACAGTACAATCCTCGAATCGTTTTAGAGCTAGAAATAGC
KU70#1*	ZA2485	GAAATTAATACGACTCACTATAGGCAGCTCTATTTCATCCGCAGGTTTTAGAGCTAGAAATAGC
KU70#2*	ZA2486	GAAATTAATACGACTCACTATAGGTCCAGCTCTATTTCATCCGCGTTTTAGAGCTAGAAATAGC
KU70#3	ZA2487	GAAATTAATACGACTCACTATAGGAACGATGAGCCGCATTCGGCGTTTTAGAGCTAGAAATAGC
KU70#4	ZA2488	GAAATTAATACGACTCACTATAGGGCAGTGTTTTGTCAAGGCGAGTTTTAGAGCTAGAAATAGC
Lig4#1	ZA2393	GAAATTAATACGACTCACTATAGGACCGGTAGCAATGGGAGCAGGTTTTAGAGCTAGAAATAGC
Lig4#2	ZA2394	GAAATTAATACGACTCACTATAGGGAATCCGGCACCGGTAGCAAGTTTTAGAGCTAGAAATAGC
Lig4#3*	ZA2395	GAAATTAATACGACTCACTATAGGCCAGCTTGAGATCTACTCTTGTTTTAGAGCTAGAAATAGC

Lig4#4* ZA2396
GAAATTAATACGACTCACTATAGGTGGCAAAGCATGGGCTTTACGTTTTAGAGCTAGAAATAGC

Lig4#5 ZA2461
GAAATTAATACGACTCACTATAGGGAGGCGGTCGAGAGTGGCGAGTTTTAGAGCTAGAAATAGC

Lig4#6 ZA2462
GAAATTAATACGACTCACTATAGGGGGGCTGTATCATCCCAAAGGTTTTAGAGCTAGAAATAGC

Lig4#7 ZA2463
GAAATTAATACGACTCACTATAGGGATGCGCGGACGTTGCCCGGGTTTTAGAGCTAGAAATAGC

Lig4#8 ZA2464
GAAATTAATACGACTCACTATAGGCAACCAGCACCTGGATGCCAGTTTTAGAGCTAGAAATAGC

Lig4#9 ZA2466
GAAATTAATACGACTCACTATAGGCCAAATTCATAAAGAAGGACGTTTTAGAGCTAGAAATAGC

Lig4#10 ZA2467
GAAATTAATACGACTCACTATAGGAGGTTCCAAATTCATAAAGAGTTTTAGAGCTAGAAATAGC

Loqs#1 ZA2437
GAAATTAATACGACTCACTATAGGGGTCGAGGATCTGAACGGACGTTTTAGAGCTAGAAATAGC

Loqs#2 ZA2438
GAAATTAATACGACTCACTATAGGGGACTGGTATCCGGTGACAAGTTTTAGAGCTAGAAATAGC

Loqs#3* ZA2505
GAAATTAATACGACTCACTATAGGAGATTCGGTTGACCGACCGGGTTTTAGAGCTAGAAATAGC

Loqs#4 ZA2506
GAAATTAATACGACTCACTATAGGGCCCTCAAGACCGAGCTGACGTTTTAGAGCTAGAAATAGC

LoqsB#1* ZA2441
GAAATTAATACGACTCACTATAGGGCCGTGACCGGTAGTGAGGCGTTTTAGAGCTAGAAATAGC

LoqsB#2 ZA2442
GAAATTAATACGACTCACTATAGGAAGGCCCTGAAGGCACGCAGGTTTTAGAGCTAGAAATAGC

LoqsB#3 ZA2503
GAAATTAATACGACTCACTATAGGAGGCGGCCAGCATTTCCGGGGTTTTAGAGCTAGAAATAGC

LoqsB#4* ZA2504
GAAATTAATACGACTCACTATAGGCGTATTCCCAGCCTCACTACGTTTTAGAGCTAGAAATAGC

LoqsB#5 ZA2589
GAAATTAATACGACTCACTATAGGAGCATTTCCGGGCGGTACGCGTTTTAGAGCTAGAAATAGC

LoqsB#6* ZA2590
GAAATTAATACGACTCACTATAGGCTTCAATCCAGCGTACCGCCGTTTTAGAGCTAGAAATAGC

LoqsB#7 ZA2591
GAAATTAATACGACTCACTATAGGAAAAGTGTCCCAGTTCCACAGTTTTAGAGCTAGAAATAGC

LoqsB#8 ZA2592
GAAATTAATACGACTCACTATAGGCCAGTTCCACAAGGCCCTGAGTTTTAGAGCTAGAAATAGC

R2D2#1 ZA2433
GAAATTAATACGACTCACTATAGGCGGAATTGGCCGGTCAAAGAGTTTTAGAGCTAGAAATAGC

R2D2#2 ZA2434
GAAATTAATACGACTCACTATAGGACCACTTCGGTTTTGGCCCTGTTTTAGAGCTAGAAATAGC

R2D2#3* ZA2507
GAAATTAATACGACTCACTATAGGTCCGCTGCTGGTGAATCCGAGTTTTAGAGCTAGAAATAGC

R2D2#4* ZA2508
GAAATTAATACGACTCACTATAGGGGAGTCTTCGTGCTCAGGACGTTTTAGAGCTAGAAATAGC

R2D2#5* ZA2583
GAAATTAATACGACTCACTATAGGCGTGCTCAGGACTGGCTTTGGTTTTAGAGCTAGAAATAGC

R2D2#6 ZA2584
GAAATTAATACGACTCACTATAGGGAGCACGAAGACTCCAATAAGTTTTAGAGCTAGAAATAGC

R2D2#7 ZA2585
GAAATTAATACGACTCACTATAGGTCACCAGCAGCGGAATTGGCGTTTTAGAGCTAGAAATAGC

R2D2#8* ZA2586
GAAATTAATACGACTCACTATAGGTCAAACTCTGGAGCATGCGAGTTTTAGAGCTAGAAATAGC

R2D2#9* ZA2587
GAAATTAATACGACTCACTATAGGTCGTCTTGTGAATTCCCCGGGTTTTAGAGCTAGAAATAGC

R2D2#10* ZA2588
GAAATTAATACGACTCACTATAGGGTTTGGAATCCTTCTTTGACGTTTTAGAGCTAGAAATAGC

Nix#1* ZA2415
GAAATTAATACGACTCACTATAGGGCAGGTTTAACGTCGCAATGGTTTTAGAGCTAGAAATAGC

Nix#2* ZA2416
GAAATTAATACGACTCACTATAGGGAACGATATCGAACAATTGCGTTTTAGAGCTAGAAATAGC

Nix#3* ZA2417
GAAATTAATACGACTCACTATAGGGTCAGCAGATATCTTGAATAGTTTTAGAGCTAGAAATAGC

Nix#4* ZA2418
GAAATTAATACGACTCACTATAGGTACCACAATGCAAGTATCATGTTTTAGAGCTAGAAATAGC

Nix#5 ZA2619
GAAATTAATACGACTCACTATAGGGAACGAAACATTAACATGTGGTTTTAGAGCTAGAAATAGC

Nix#6 ZA2620
GAAATTAATACGACTCACTATAGGCGGTCTAAATTCAGAAAGCCGTTTTAGAGCTAGAAATAGC

Nix#7* ZA2621
GAAATTAATACGACTCACTATAGGTTAGATTTGTGGAGAAATCCGTTTTAGAGCTAGAAATAGC

Nix#8* ZA2622
GAAATTAATACGACTCACTATAGGCTTGAATAAGGCAAACTTAGGTTTTAGAGCTAGAAATAGC

[a] T7 promoter indicated in bold, sgRNA target site is underlined.

* indicates sgRNA targets the reverse strand.

List of HRMA assay primers

Primer	Sequence	HRMA Assay	CRISPR/TALEN target within assay
KMF1	CCATGGTTCCCTTCTACGG	KMO-Assay 1	KMO519, KMO519*
KMR1	TCACTAAACTCAGCCAGTATCCTAT		
ZA2206	TGCCCGTAGAAGGGAAC	KMO-Assay 2	KMO447, KMO468, KMO460
ZA2210	TTCAAGACCAGGCCTCAATC		
ZA2207	AGTATGTCCGGAAGAACTTCAACAAATC	KMO-Assay 3	KMO304, KMO281*
ZA2211	CACTTGGACGGTGACGCT		
ZA2477	GAGTTGTGTTCTACAACACG	ku70-Assay 1	ku70#1*, ku70#2*
ZA2479	TTTCTTCGGGACTACCAAT		
ZA2481	ATCCAGCATTGTCCTGTTC	ku70-Assay 2	ku70#3, ku70#4
ZA2483	ATCAAAACTATGTTGATGTCCAG		
ZA2390	ATCGAGGCCCGTAAGTTGT	lig4-Assay 1	lig4#1, lig4#2
ZA2391	AACGTCCGCGCATCAACGTG		
ZA2273	GTGGGAATGGCTGTTCGATTA	lig4-Assay 2	lig#3*, lig#4*
ZA2275	GTCCTTCTTCAGCATGCCATC		
ZA2563	GGTTCCCCGACTTCTTCCA	lig4-Assay 3	lig4#5, lig4#6
ZA2564	GGATTATACTGAAGAATCTGCGGC		
ZA2469	GGTAGCAATGGGAGCAGCG	lig4-Assay 4	lig4#7, lig4#8
ZA2470	ATGAGCTCGTCCTGCGTTT		
ZA2471	AATTCTGGCTGGAAACTAA	lig4-Assay 5	lig4#9, lig4#10
ZA2472	CTGTTCCTGGAGAAGTATT		
ZA2435	AGATGAGTAAGACGGATTTGC	loqs-Assay 1	loqs#1, loqs#2, loqs#3*
ZA2436	ACCGATTTCTTCGCCAC		
ZA2501	CGGTGAGCAAGCAGGATG	loqs-Assay 2	loqs#4
ZA2502	CCGAGATCGGTGTCTTCATGT		
ZA2439	GCGGTACGCTGGATTGA	loqsB-Assay 1	loqsB#1*, loqB#2, loqB#4*, loqB#7, loqB#8

ZA2440	CAGTTGGCGCAGAGTTTC		
ZA2495	TGTGTGTTTTTCCCTATCTAT	loqsB-Assay 2	loqB#3, loqB#5, loqB#6*
ZA2496	GGGACACTTTTTGGCCGT		
ZA2430	CAACTTATAGGCAGCGTCAT	R2D2-Assay1	R2D2#1, R2D2#2, R2D2#3*, R2D2#7, R2D2#10*
ZA2431	ATCGACGGAGGAAACCC		
ZA2497	AAGTTTGTACCTTGTGGTGTAG	R2D2-Assay2	R2D2#4*, R2D2#5*, R2D2#6
ZA2498	CACACACAGCTCTTGCAG		
ZA2581	TGCCCGAATTCAACTGTGT	R2D2-Assay3	R2D2#8*, R2D2#9*
ZA2582	CCTGTTTGGCCCCTTTC		
ZA2419	AATACGGCGAAACATGTAA	Nix-Assay A	Nix#1*, Nix#2*
ZA2420	CACAAAGACTTGTCTACAC		
ZA2421	CGGCAACACGATATTAATAG	Nix-Assay B	Nix#3*, Nix#4*, Nix#8*
ZA2422	CTCAGCCATTGATGTCTTAC		
ZA2617	TCTTTAGCTACTAAGCCCT	Nix-Assay C	Nix#5, Nix#6, Nix#7*
ZA2618	TCCAATAGAGCGATTATACAA		
Dcr2ex5P1	GGTTATCTCTCGGTTAAACACA	Dcr2 Exon5 Assay 1	TALEN dcr2 exon5
Dcr2Ex5P1R	CATAGGATGTTCTCCTACACCA		
Dcr2Ex8P1F	AATACCCATAGACTGCAAATACCA	Dcr2 Exon8 Assay 1	TALEN dcr2 exon8
Dcr2Ex8P1R	AATCCTTCCAATGACGGAAATACA		
Ago2Ex2P2F	TTCGCATTCGTCGTCACC	Ago2 Exon2 Assay 1	TALEN ago2 exon2
Ago2Ex2P2R	TCTTGATCTTGCTGAAATCCTCC		
Ago2Ex3P2F	TGAGCTGTCCTACCGCA	Ago2 Exon3 Assay 1	TALEN ago2 exon3
Ago2Ex3P2R	TGAAGTTCATGTTGTCAGCCC		
ZA2904	CATGATTCCTTACCTGAAGATTTCAAGA	R2D2 1st off-target assay	(R2D2#6)
ZA2905	AGACATGGCTTGATATTTACCCATT		
ZA2906	GCTGCTGCTGCTACCGA	R2D2 2nd off-target assay	(R2D2#6)
ZA2907	CGAGTCTAATTTACTTCCTGGTGTC		

ZA2900	GCACTGGGCCTTGCGGAAAC	Ku70 1st off-target assay	(Ku70#3)
ZA2901	GTCGGATTACATTGCCGTCAAGGAGA		
ZA2902	ATTCTTACGTGATCCACCACGG	Ku70 2nd off-target assay	(Ku70#3)
ZA2903	AAATCGCACAGTGCACAAAGC		
ZA2932	ATGCCAATCGGGTCACATTCTC	Loqs 1st off-target assay	(Loqs#3*)
ZA2933	CACACGTCTGTTCGACCAGTT		
ZA2934	ACTAAGCGAACGTCAAACATGATAAA	Loqs 2nd off-target assay	(Loqs#3*)
ZA2935	TCGTTCTTGCGTTACGTCACTC		
ZA2936	ATAGGGTAAATACCATTCTTCACCAA	Nix 1st off-target assay	(Nix#8*)
ZA2937	TTGATAACTCTTAGGTATTTCTTCACG		
ZA2938	AGCTATTTGAATCTATGGATAAGAGTTGT	Nix 2nd off-target assay	(Nix#8*)
ZA2939	GCCATGCCAAATTACTATGGGAA		

List of dsRNA primers.

dsRNA target	Primer	Sequence
lig 4	ZA2456	taatacgactcactatagggCGGCATCCGGGTAAAAACTCTGC
	ZA2459	ggaaaaaaaGCTTTACAGGATTGAATAATCGA
ku70#1	ZA2341	taatacgactcactatagggTTTCGCTGGGTGTCAACATTTATTCC
	ZA2342	ggaaaaaaaaaCTTGATTTTCTTCGCTACTGACTTGA
ku70#2	ZA2474	taatacgactcactatagggCATGAGAAACAAGATCATCAG
	ZA2476	ggaaaaaaaGATCTTCGTCGGCTACCGTACA
ku80	ZA2339	taatacgactcactatagggTTCACCCAAAAGCTCCCATACC
	ZA2340	ggaaaaaaaCTCCCAATCAATCCCAAATCGTT
GFP	ZA2351	taatacgactcactatagggATGGTGAGCAAGGGCGAGGAGC
	ZA2352	ggaaaaaaaaTCTTGAAGTTCACCTTGATGCCGTT

List of highly active sgRNA targets and their most likely off target sites.

sgRNA#	Locus	Sequence	Genomic coordinates
KMO460	AAEL008879	CGGCAAGGCGGTGATCATTGG	supercontig 1.354: 1099926-1099940
KMO460	1st off target site	CGGCAAGCCGGTGATCATGGG	supercontig 1.892: 349339-349353
KMO460	2nd off target site	CGTCAAGGCGGTGATGATCGG	supercontig 1.1178: 54005-54019
R2D2#6	AAEL011753	GAGCACGAAGACTCCAATAACGG	supercontig1.608: 70829-70843
R2D2#6	1st off target site	CTACGAATAGACTCCAATAATGG	supercontig1.633: 211688-211702
R2D2#6	2nd off target site	TCTGCTGTAGACTCCAATAAGGG	supercontig1.219: 895738-895752
ku70#3	n/a	AACGATGAGCCGCATTCGGCCGG	supercontig 1.240: 506077-506091
ku70#3	1st off target site	AAATATCTGCCGCATTCGGCTGG	supercontig 1.887: 21114-21128
ku70#3	2nd off target site	GTTCATTAGCCGCATTCGGCAGG	supercontig 1.99: 805419-805433
loqs#3	AAEL008687	AGATTCGGTTGACCGACCGGGGG	supercontig 1.342: 1272241-1272255
loqs#3	1st off target site	CAATTGTATTGACCGACCGGGGG	supercontig 1.244: 930696-930710
loqs#3	2nd off target site	AAAGATCCTTGACCGACCGGGGG	supercontig 1.73: 2810642-2810656
Nix#8	n/a	CTTGAATAAGGCAAACTTAGTGG	n/a
Nix#8	1st off target site	GGAACACAAGGCAAACTTAGTGG	supercontig 1.105: 66194-66208
Nix#8	2nd off target site	GTTGAAAAAGGCAAACTGAGTGG	supercontig 1.510: 228086-228100